

WHAT IS CLAIMED:

1. A method of selecting phage that encode a target binding protein from a plurality of display phage, the method comprising:

- a) forming a mixture comprising a plurality of diverse display phage, a target, and a support, wherein each phage of the plurality displays a heterologous protein component on its surface and each phage includes a nucleic acid encoding the heterologous protein component, the heterologous protein component being a member of a set of diverse protein components;
- b) forming phage-immobilized target complexes, each of which comprises a phage from the plurality which binds the target and the target immobilized to the support;
- c) separating phage that do not bind to the target from the phage-immobilized target complexes;
- d) contacting host cells with the phage-immobilized target complexes so that the host cells are infected by phage from the phage-immobilized target complexes to yield a first population of infected cells;
- e) producing replicate phage from the infected cells in the presence of the target thereby forming replicate phage-immobilized target complexes;
- f) separating replicate phage that do not bind to the target from the replicate phage-immobilized target complexes; and
- g) contacting host cells with the replicate phage-immobilized target complexes so that host cells are infected with the replicate phage to yield a second population of infected cells.

2. The method of claim 1 further comprising recovering the second population of infected cells.
3. The method of claim 1 further comprising recovering phage produced by the second population of infected cells.
4. The method of claim 1 further comprising repeating steps e) to g) at least once.
5. The method of claim 1 wherein steps a) to g) are conducted in the same vessel.
6. The method of claim 1 wherein steps d) to e) occur in the same vessel.
7. The method of claim 1 wherein steps b) to g) are conducted without addition of the target.
8. The method of claim 1 wherein step e) comprises supplying the mixture in which the replicate phage are produced with an additional amount of the target.
9. The method of claim 1 wherein, during step e), fewer than 5000 progeny phage are produced for each phage that infects one of the host cells.
10. The method of claim 1 wherein step e) is completed in less than 4 hours.
11. The method of claim 1 wherein, during step e), the host cells divide less than seven times.

12. The method of claim 1 wherein time between the contacting d) and the separating f) is less than 240 minutes.

13. The method of claim 1 wherein the diverse set of protein components consists of between 10^3 and 10^{12} different protein components.

14. The method of claim 1 wherein the producing comprises a change in temperature.

15. The method of claim 1 wherein each diverse phage of the plurality comprises genes sufficient for phage replication in a host cell.

16. The method of claim 1 wherein each diverse phage of the plurality is a phagemid, and the producing e) comprises contacting helper phage to the host cells.

17. The method of claim 1 wherein a competing ligand is present during an interval of the producing e).

18. The method of claim 1 wherein during step e) and/or g) the host cells are cells of a mutator strain.

19. A method of selecting phage that encode a target binding protein from a plurality of display phage, the method comprising:

a) forming a mixture comprising a plurality of diverse display phage, a first target, and a support, wherein each phage of the plurality displays a heterologous protein component on its surface and each phage includes a nucleic acid encoding the heterologous protein component, the heterologous protein component being a member of a set of diverse protein components;

b) forming phage-immobilized target complexes, each of which comprises a phage from the plurality which binds the first target and the first target immobilized to the support;

c) separating phage that do not bind to the first target from the phage-immobilized target complexes;

d) contacting host cells with the phage-immobilized target complexes in a suitable growth medium so that the host cells are infected by phage from the phage-immobilized target complexes to yield a first population of infected cells;

e) producing replicate phage from the infected cells in the presence of a second target thereby forming replicate phage-immobilized target complexes that include replicate phage that bind to the second target and the second target immobilized to a support;

f) separating replicate phage that do not bind to the second target from the replicate phage-immobilized target complexes; and

g) contacting host cells with the replicate phage-immobilized target complexes so that host cells are infected with the replicate phage to yield a second population of infected cells.

20. A method of identifying members of a bacteriophage library that have a desired binding property, the method comprising:

- (a) providing a bacteriophage library that comprises a plurality of bacteriophage members;
- (b) selecting a subset of the bacteriophage members;
- (c) infecting host cells with the members of the subset;
- (d) amplifying members of the subset under at least one of the following conditions:
 - (1) fewer than 5000 progeny phage are produced for each input phage;
 - (2) less than 4 hours elapses; and
 - (3) the host cells divide less than 6 times; and
- (e) selecting a subset of the amplified members, thereby identifying the desired members of the bacteriophage library.

21. The method of claim 20 wherein the amplifying (d) occurs in the presence of a target, and step (e) comprises selecting amplified members that bind to the target.

22. The method of claim 21 wherein the target is a compound that is immobilized during the amplifying (d).

23. The method of claim 20 wherein step (b) comprises contacting the bacteriophage library to a target and a solid support, immobilizing members of the library that bind to the target, and separating members of the library that bind to the target from members of the library that do not bind to the target.

24. A method of selecting a nucleic acid that encodes a binding protein from a library of display phage, the method comprising:

a) providing a library of phage that each have a heterologous protein component that is diverse among the phage of the plurality, physically attached to the phage, and accessible;

b) contacting phage of the library to a target;

c) performing one or more cycles of:

i) forming phage-immobilized target complexes, each of which comprises (1) a phage that binds to the target by its heterologous protein component and (2) the target immobilized to a support,

ii) separating phage that do not bind to the target from the phage-immobilized target complexes,

iii) contacting phage from the phage-immobilized target complexes with host cells so that the host cells are infected by the phage from the phage-immobilized target complexes, and

iv) producing phage from the infected cells in the presence of the target, the produced phage being replicates of phage that bind to the target; and

d) recovering the nucleic acid encoding the heterologous protein component of one or more produced phage that bind to the target, thereby selecting a nucleic acid that encodes a binding protein for the target.

25. The method of claim 24 wherein conditions of the separating in step ii) vary in stringency during the cycles.

26. The method of claim 24 wherein at least two cycles are performed.

27. The method of claim 26 wherein at least three cycles are performed.

28. The method of claim 24 wherein each cycle is completed in less than 8 hours.